PANTOTHENIC ACID AND THE UTILIZATION

OF GLUCOSE BY LIVING AND CELL-FREE SYSTEMS

Approved:

Roger J. Williams

Henry R. Henze

H. L. Lochte

Robbin C. Anderson

W. A. Selvig

F. M. Lewis

A. T. Schwabacher

Approved:

A. P. Brogan

Dean of the Graduate School

PANTOTHENIC ACID AND THE UTILIZATION OF GLUCOSE BY LIVING AND CELL-FREE SYSTEMS

DISSERTATION

presented to the faculty of the graduate school of
The University of Texas in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

Peyton Clark Teague, B.S., M.S.

Austin, Texas
June, 1942

Acknowledgment

The author wishes to express his appreciation to Dr. Roger J. Williams for suggesting this problem and directing the research, to the University of Texas for the grant of a University Advanced Fellowship for the years 1939-40 and 1940-41, and to Drs. E. E. Snell and C. M. Lyman for valuable suggestion and assistance during the course of this work.

March, 1942

TABLE OF CONTENTS

I

INTRODUCTION

INTRODUCTION

Pantothenic acid was discovered by Williams and coworkers[1,2,3,4] in their studies on the physiological principle required for the growth of yeast. Work in Williams' laboratory showed that β-alanine[5,6] is a constituent of the molecule and partially elucidated the structure of the remaining portion[7,8,9]. The final

---

[1]R. J. Williams, E. Bradway, J. Amer. Chem. Soc., 53: 783 (1931).

[2]R. J. Williams, J. H. Truesdail, Ibid., 53: 4171 (1931).

[3]R. J. Williams, C. M. Lyman, G. H. Goodyear, J. H. Truesdail, Ibid., 54: 3462 (1932).

[4]R. J. Williams, C. M. Lyman, G. H. Goodyear, J. H. Truesdail, D. Holaday, Ibid., 55: 2912 (1933).

[5]R. J. Williams, E. Rohrmann, Ibid., 58: 695 (1936).

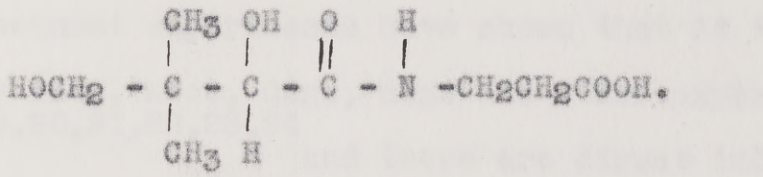
[6]H. H. Weinstock, Jr., H. K. Mitchell, E. F. Pratt, R. J. Williams, Ibid., 61: 1421 (1939).

[7]R. J. Williams, J. H. Truesdail, H. H. Weinstock, Jr., E. Rohrmann, C. M. Lyman, C. H. McBurney, Ibid., 60: 2719 (1938).

[8]R. J. Williams, H. H. Weinstock, Jr., E. Rohrmann, J. H. Truesdail, H. K. Mitchell, C. E. Meyer, Ibid., 61: 454 (1939).

[9]H. K. Mitchell, H. H. Weinstock, Jr., E. E. Snell, S. Stanbery, R. J. Williams, Ibid., 62: 1776 (1940).

identification[10,11,12,13] was obtained by collaboration with the Merck Research Laboratories and pantothenic acid was shown to have the structure



Long before pantothenic acid was isolated it was shown to be present in a wide variety of organisms[14,15]. In fact every organism tested was shown to contain it. This led to the early suggestion that it might be of universal importance. The substance was shown to be required in the nutrition, not only of yeast, but of other microorganisms, and to stimulate the growth of green plants.[16] The final proof that pantothenic acid is a

---

[10] R. J. Williams and R. T. Major Science, 91: 246 (1940).

[11] R. J. Williams, H. K. Mitchell, H. H. Weinstock, Jr., E. E. Snell, J. Amer. Chem. Soc., 62: 1784 (1940).

[12] E. T. Stiller, J. C. Keresytesy, J. Finkelstein, Ibid., 62: 1779 (1940).

[13] E. T. Stiller, S. A. Hanis, J. Finkelstein, J. C. Keresytesy, K. Folkers, Ibid., 62: 1785 (1940).

[14] R. J. Williams, C. M. Lyman, G. H. Goodyear, J. H. Truesdail, op. cit.

[15] R. J. Williams, C. M. Lyman, G. H. Goodyear, J. H. Truesdail, D. Holaday, op. cit.

[16] R. J. Williams, E. Rohrman, Plant Physiol., 10: 559 (1935).

vitamin was furnished simultaneously by Woolley, Waisman, and Elvehjem[17] and by Jukes[18], when it was shown to be identical with the "chick antidermatitis factor."

Subsequent experiments have shown that it is required by rats, mice, dogs, hogs and other experimental animals[19,20,21,22,23,24] and there are direct indications that it is also required by human beings[25].

Since the discovery of the vitamin properties of pantothenic acid, much interest has been aroused as to its biochemical function. However, prior to this time, Williams and coworkers had already done some work bearing

---

[17] D. W. Wooley, H. A. Waisman, C. A. Elvehjem, J. Amer. Chem. Soc., 61: 997 (1939).

[18] T. H. Jukes, Ibid., 61: 976 (1939).

[19] Y. Subbarow, G. H. Hitchings, Ibid., 61: 1615 (1939).

[20] J. J. Oleson, D. W. Woolley, C. A. Elvehjem, Proc. Soc. Exp. Biol., 42: 151 (1939).

[21] L. R. Richardson, A. G. Hogan, Ibid., 44: 585 (1940).

[22] J. M. McKibbin, R. J. Madden, S. Block, C. A. Elvehjem, Amer. J. Phys., 128: 102 (1939).

[23] J. M. McKibbin, S. Block, C. A. Elvehjem, Ibid., 130: 365 (1940).

[24] E. H. Hughes, J. Nutrition, 17: 527 (1939).

[25] T. D. Spies, S. Stanbery, R. J. Williams, T. H. Jukes, S. H. Babcock, J. Amer. Med. Ass., 115: 523 (1940).

on this problem. They showed that it stimulated the deposition of carbohydrate by green plants without affecting their nitrogenous constituents[26]. The glycogen storage of yeast was also found to be increased by incubating with pantothenic acid[27]. Yeast grown on a medium deficient in pantothenic acid possessed a low fermenting ability which was greatly increased by the addition of pantothenic acid[28]. Such yeast was found to have a much lower pantothenic acid content than normal yeast. The rate of fermentation of sucrose by dialyzed yeast maceration juice was also observed to be slightly accelerated by the addition of calcium pantothenate. It was likewise found to have a stimulating effect on the respiration of apple and potato tissues and indications were obtained of a similar effect on certain animal tissues. These experiments indicated that pantothenic acid was involved, probably as a coenzyme, in some stage of carbohydrate metabolism.

Carbohydrate metabolism in different tissues and organisms varies considerably in the nature of the

---

[26] E. H. McBurney, W. B. Bollen, R. J. Williams, Proc. Nat. Acad. Sc., 21: 301 (1935).

[27] R. J. Williams, W. A. Mosher, E. Rohrman, Biochem., J., 30: 2036 (1936).

[28] E. F. Pratt, R. J. Williams, J. Gen. Physiol., 22: 637 (1939).

initial and final products. However, recent work has shown that many of the intermediate processes are identical and that the whole process takes place through the influence of a complicated series of enzymes and coenzymes, which are similar but not identical in all cases. The type of carbohydrate breakdown which has received the greatest amount of attention is alcoholic fermentation by yeast. The work of Meyerhof, Parnas, Cori and others has led to the modern theory which is outlined below[29,30,31,32,33,34,35]. The composition of the enzymes, since these are complex substances, is not known. A number of simple substances which act as coenzymes include: cozymase or coenzyme I (Co), adenylic acid, adenosine triphosphate (adenylpyrophosphate, A. T. P.), cocarboxylase (thiamin pyrophosphate).

The following series of equations summarizes the

---

[29] F. F. Nord, Chem. Rev., 26: 423 (1940).

[30] W. H. Chambers, S. B. Barker, Ann. Rev. Biochem., 9: 253 (1940).

[31] C. F. Cori, G. T. Cori, Ibid., 10: 151 (1941).

[32] M. Kerly and M. C. Bourne, Biochem. J. 34: 1503 (1940).

[33] O. Meyerhof, E. Perdigon, Enzymologia, 8: 353 (1940).

[34] D. Herbert, H. Gordon, V. Subrahmanyan, D. E. Green, Biochem. J., 34: 1070 (1940).

[35] I. K. Parnas, Enzymologia, 5: 172 (1938).

conversion of glucose into alcohol and carbon dioxide. The names of the coenzymes are placed above the arrows of the successive reactions in which they are known to act except in the case of the phosphorylation reactions, in which case they are written in the equations. The phosphorylation reactions are written with adenosine triphosphate being converted into adenosine diphosphate, although it is known that in some cases adenosine diphosphate can itself act as a phosphorylating coenzyme and be converted into adenylic acid.

(1) Glucose + A. T. P. $\rightleftharpoons$ glucose-6-phosphate + adenosine diphosphate (A. D. P.).

(2) Glucose-6-phosphate $\rightleftharpoons$ fructose-6-phosphate.

(3) Fructose-6-phosphate + A. T. P. $\rightleftharpoons$ fructose-1, 6-diphosphate A. D. P.

(4) Fructose-1,6-diphosphate $\rightleftharpoons$ dihydroxyacetone phosphate + 3-glyceraldehyde phosphate.

(5) Dihydroxyacetone phosphate $\rightleftharpoons$ 3-glyceraldehyde phosphate.

(6) 3-Glyceraldehyde phosphate + A. T. P. $\rightleftharpoons$ 1, 3-diphosphoaldotriose A. D. P.

(7) 1,3-Diphosphoaldotriose + acetaldehyde + A. D. P. $\overset{Co}{\rightleftharpoons}$ ALCOHOL + 3-phosphoglyceric acid + A. T. P.

(8) 3-Phosphoglyceric acid $\rightleftharpoons$ 2-phosphoglyceric acid.

(9) 2-Phosphoglyceric acid $\rightleftharpoons$ 2-phosphopyruvic acid.

(10) 2-Phosphopyruvic acid + A. D. P. $\rightleftharpoons$ pyruvic acid + A. T. P.

(11) Pyruvic acid $\overset{\text{cocarboxylase}}{\rightleftharpoons}$ acetaldehyde + CARBON DIOXIDE.

↓ To equation (7)

Muscle glycolysis differs chiefly from alcoholic fermentation in that the final product is lactic acid instead of alcohol and carbon dioxide. The lactic acid may in turn be oxidized to carbon dioxide and water. Willstätter[36] believes that carbohydrate metabolism of yeast and muscle proceeds through the formation of glycogen. In this case the primary product is glucose-1-phosphoric acid which is then converted to glucose-6-phosphoric acid. Macfarlane and other[37,38] contend that, although yeast can metabolize glycogen directly, the principal fermentation substrate is glucose. Carbohydrate metabolism in brain tissue is believed to differ from that of muscle in that glucose and not glycogen is believed to be the substrate.

The object of the experiments reported in this thesis was to observe the effect of pantothenic acid on the respiration of various tissues and cell-free preparations and to investigate the role of pantothenic acid in the metabolism of glucose. The work with deficient yeast and yeast maceration juice was repeated and extended. Since the tissues of chicks grown on a diet deficient in panto-

---

[36] R. Willstatter, M. Rohdewald, Z. Physiol. Chem., 247: 269 (1937).

[37] M. G. Macfarlane, Biochem. J. 33: 565 (1939).

[38] T. Goda, Biochem. Z. 298: 431 (1938).

thenic acid are low in pantothenic acid[39], deficient brain and muscle tissues also served as basal tissues on which the effects of pantothenic acid could be studied.

---

[39] E. E. Snell, D. Pennington, R. J. Williams, J. Biol. Chem. 133: 559 (1940).

II

EFFECT ON MACERATION JUICE

EFFECT ON MACERATION JUICE

Preliminary experiments were run to confirm the effect of pantothenic acid on the fermentation of glucose and sucrose by Gebrüder-Mayer yeast deficient in pantothenic acid. The yeast was grown on a medium containing sucrose, asparagin, inositol, minerals and a small amount of rice bran extract (100 mgm. per liter). The rice bran extract furnished enough pantothenic acid to insure an appreciable crop of yeast but not enough to prevent the yeast from becoming deficient. Addition of pantothenic acid was shown to greatly increase the rate of fermentation of both sucrose and glucose by this deficient yeast as measured by both the rate of carbon dioxide evolution and the total gas exchange.

All respiration experiments were carried out in a fourteen unit Warburg-Bancroft microrespirometer[40].

Maceration juice was prepared from Fleischmann's baker's yeast. The procedure used was essentially that of Lebedev[41]. Washed yeast was dried at room temperature under an electric fan. A quantity, not over fifty grams, of this dried yeast was mixed with three times its volume

---

[40]Standard procedures for measurement of respiration may be found in Dixon's Manometric Methods, London, Cambridge University Press, 1934.

[41]A. V. Lebedev, Ann. Inst. Pasteur, 26: 8 (1912).

of water and incubated at 37° for two hours. The cells were then removed from the juice either by filtration or centrifugation.

This juice was freed of its major portion of pantothenic acid in one of two ways: (1) by dialysis (2) by precipitating the active enzymes with acetone and redissolving the precipitate in 1% sodium chloride solution. It was necessary to carry out the dialysis at low temperatures (0-10°) because the maceration juice loses its activity rapidly at room temperature.

The acetone precipitation was carried out by pouring the juice with constant stirring into about twenty-five times its volume of acetone. The precipitate was filtered off and dried either in air or in a vacuum desiccator. It was then made up to the original volume of the juice by dissolving in either sodium chloride or potassium dihydrogen phosphate solution. The acetone denatured a portion of the proteins but did not seem to affect appreciably the activity of the enzymes.

Either of these procedures gives a preparation containing considerably less pantothenic acid than normal maceration juice. The pantothenic acid was determined using the method of Pennington, Snell, and Williams[42].

---

[42] D. Pennington, E. E. Snell, R. J. Williams, J. Biol. Chem. 135: 213 (1940).

For normal maceration juice the average was about 55γ per cc. A number of experiments served to show that over sixty percent of the pantothenic acid was removed from the juice by dialyzing eight hours, and over 90% by one precipitation with acetone.

It was observed that additional pantothenic acid, which was not detected in tests on the autolized juice, could be liberated by enzymatic action. One preparation in which the acetone precipitation and re-solution in sodium chloride solution were repeated twice, contained only 0.45γ per cc. of "free" pantothenic acid. Enzymatic action liberated from this juice an additional 5-6γ per cc. of "bound" pantothenic acid. The determination of "bound" pantothenic acid was carried out by incubating a portion of the juice with homogenized fresh muscle tissue and allowing the autolytic enzymes of the muscle tissue to act on the yeast juice. The difference between the pantothenic acid content of this mixture and of the fresh muscle tissue itself was taken as the sum of the "free" and "bound" pantothenic acid in the yeast juice.

Maceration juice is known to be deficient in adenosine-triphosphate [43]. In order to bring about fermentation the juice must be supplied with this necessary

---

[43] C. Lutwak-Mann, T. Mann, Biochem. Z. 281: 140 (1935)

coenzyme. The easiest way to supply this deficiency (indirectly) is to add fructose-1,6-diphosphate and adenylic acid. The enzymes of the juice are capable of transferring the phosphate from the sugar phosphate to adenylic acid. Fructose-1,6-diphosphate was prepared and purified according to the method of Robison and Morgan[44]. A small amount of this preparation rendered active an otherwise inactive juice. The fructose diphosphate alone was not fermented by the juice.

Fermentation of glucose: A number of experiments were performed to determine the effect of pantothenic on the fermentation of glucose by the dialyzed and acetone precipitated yeast juices. No effect due to pantothenic acid was observed. In a typical experiment maceration juice was prepared as usual and dialyzed for 8 3/4 hours in a cellophane sack at 0-10° against 0.9% sodium chloride solution. One cc. of this juice was then pipetted into each of eight flasks containing 1 cc. of 0.4 M. glucose, 1 cc. of KH_2PO_4 (8 g. per liter), 10γ of adenylic acid, 10γ of coenzyme I, 0.1 cc. of salt solution (0.025 M. $MgSO_4$, 0.05M. $MnSO_4$, and 0.2M. $(NH_4)_2SO_4$), and 7 mg. of sodium fructose-diphosphate. The flasks were then connected to the manometers, placed

---

[44] R. Robison, W. T. J. Morgan, Biochem. J., 24: 119 (1930).

in the constant temperature water bath and shaken for fifteen minutes to reach equilibrium. The stopcocks were then closed and the carbon dioxide evolution noted. Table I gives the results of this experiment.

Table I

Fermentation of Glucose by Dialyzed Maceration Juice

Calcium pantothenate	0	0	0	0.1γ	0.1γ	0.1γ	10γ	10γ
Mm^3 of CO_2 per hour	368	379	375	356	358	374	351	379

Results with acetone precipitated maceration juice were exactly similar to those given above for dialyzed maceration juice.

Decarboxylation of pyruvic acid: Although it is generally accepted that no coenzyme other than cocarboxylase is required for the decarboxylation of pyruvic acid, several experiments were carried out to test the possibility of pantothenic acid having some effect on this stage of the fermentation reaction. To each Warburg flask was added 1 cc. of the acetone precipitated enzyme in KH_2PO_4 solution, 1 cc. of 0.4M sodium pyruvate, and 1 cc. of water containing the indicated amount of pantothenic acid. The pantothenic acid was placed in the

side arm of the flask and added after equilibrium had been reached. The results of this experiment are given in table II.

Table II

Decarboxylation of Pyruvic Acid

Pantothenic acid added.	0	0	0.01γ	0.1γ	1γ	8γ	40γ
Mm^3 of CO_2 evolved per hour.	77.5	87	74	78	80.5	80.5	79

A number of similar experiments were run. The results were the same whether the pantothenic acid was placed directly in the flask or added from the side arm after the equilibrium period. No effect due to pantothenic acid was observed.

Phosphorylation of glucose: The effect of pantothenic acid on the rate of phosphorylation of glucose by dialyzed maceration juice was determined. The calcium salt of pantothenic acid was used and controls containing calcium chloride were included since calcium ion can to some extent replace magnesium in the phosphorylation enzyme. Inorganic phosphate was determined according to the method of Briggs[45] with the Evelyn photoelectric

---

[45] A. P. Briggs, J. Biol. Chem., 59: 255 (1924).

colorimeter. The decrease in inorganic phosphate in the solution was assumed to be the amount esterified with glucose.

In a typical experiment freshly prepared maceration juice was dialyzed for eight hours at 0-10° against 1% sodium chloride solution. The test was carried out in ordinary bacteriological test tubes. Into each tube was placed 1 cc. of glucose, 1 cc. of KH_2PO_4 (8 gm. per liter), 1 cc. of water containing 3.5 mgm. of sodium hexose-diphosphate, 0.1 cc. of salt solution (0.025M $MgSO_4$, 0.05M $MnSO_4$, and 0.2M $(NH_4)_2 SO_4$), and 0.1 cc. of water containing the desired amount of pantothenic acid. The tubes were then placed in the 37 incubator. After the indicated time interval the tubes were removed, 1cc. of 20% trichloroacetic acid added to precipitate the proteins, and the volume made up to 10 cc. This was then filtered and inorganic phosphate in the filtrate was determined. The results are given in Table III.

Table III

Phosphorylation of Glucose by
Dialyzed Maceration Juice

Calcium pantothenate added.	Initial	10 Min.	30 Min.	60 Min.	180 Min.
0	1.16	1.12	0.89	0.56	0.48
1γ	---	1.09	0.92	0.56	0.47
10γ	---	1.12	---	0.56	0.48
100γ	---	1.10	0.92	0.57	0.50
15γ $CaCl_2$	---	1.10	0.91	0.57	0.50

Figures represent mgm. of phosphorus present as inorganic phosphate.

Several preparations of maceration juice dialyzed for various lengths of time were tested but in no case could any effect of calcium pantothenate be observed which was not attributable to the calcium ion.

These experiments with maceration juice indicate that pantothenic acid does not function as a typical dissociable coenzyme for the enzyme systems involved in the fermentation of glucose.

III

EFFECT ON LIVE YEAST

EFFECT ON LIVE YEAST

The large effect of pantothenic acid upon the fermentation of glucose by yeast deficient in pantothenic acid is apparently in contradiction to the fact that pantothenic acid showed no effect on the fermentation by maceration juice from which most of the pantothenic acid had been removed. This apparent contradiction suggested that pantothenic acid may act only in a combined form. If this is the case one would expect the accelerating action of the pantothenic acid on the deficient yeast to be accompanied by a parallel binding of the pantothenic acid in the yeast. The following experiment was performed to see if this binding takes place.

Binding of pantothenic acid by deficient yeast:

Gebrüder-Mayer yeast was grown from a heavy seeding in a medium containing sucrose, asparagin, salts, thiamin, nicotinic acid, riboflavin, biotin, and inositol but no pantothenic acid. A twelve-fold increase was obtained. These deficient cells were centrifuged off and washed with KH_2PO_4 solution before being used. A preliminary experiment using two milligram quantities of this yeast showed that an appreciable increase in CO_2 evolution occurred on the addition of as little as 0.1γ of pantothenic acid.

For the determination of the pantothenic acid bound by the deficient yeast 50 milligram quantities of yeast were used. These were incubated for four hours at 30° with 5 cc. of KH_2PO_4 solution (4 mg. per liter), 5 cc. of 0.4M glucose solution and 1 cc. of the indicated amount of pantothenic acid. The cells were then centrifuged down, washed three times with 1% NaCl solution, and finally suspended in 10 cc. of acetic acid-sodium acetate buffer solution (p^H = 4.5). One-half of each sample was steamed for ten minutes then autoclaved for fifteen minutes at 15 lbs. pressure. The hot water extract was used for the determination of the free pantothenic acid in the cells. The other half of each sample was incubated for 36 hours at 37° C. with a mixture of clarase and claroid[46]. This portion was used for the determination of the total pantothenic acid in the cells. The difference between these quantities was then taken as the amount of "bound" pantothenic acid.

---

[46]This enzyme treatment has been standardized in this laboratory for the liberation of pantothenic acid bound in tissues. Unpublished work.

Table IV

Pantothenic Acid Bound by Deficient Yeast

Pantothenic acid added.	Free pantothenic acid in 50 mgm. of deficient yeast.	Pantothenic acid bound by 50 mgm. of deficient yeast.
0	0.03 γ	0.0[a]
0.5 γ	0.06 γ	0.30 γ
5 γ	0.28 γ	0.68 γ
50 γ	0.30 γ	0.74 γ

[a]Too small to determine.

It is evident from these data that the increase in the rate of fermentation by the deficient yeast is accompanied by a parallel "binding" of the added pantothenic acid in the cell, probably to form an integral part of some enzyme. Since the medium used contained no nitrogen from which proteins might be synthesized it seems likely that the protein was already pre-formed in the cell and needed only the addition of pantothenic acid to complete the enzyme system. This would explain the absence of acceleration of fermentation upon the addition of pantothenic acid to the treated yeast juices. Commercial (Fleischmann's) yeast is grown in medium relatively rich in pantothenic acid. In this instance any of the enzyme

protein formed would probably be combined with pantothenic acid and none of the pantothenic acid-free protein would be present in the yeast juice. If this were the case, then addition of extra pantothenic acid would be expected to have no effect.

Effect of pantoyl taurine on fermentation by deficient yeast: Snell has prepared the sulfonic acid analogue of pantothenic acid, pantoyl taurine[47,48]. He has shown that this substance acts antagonistically towards the stimulating effect of pantothenic acid on the growth of yeast and other microorganisms. Several experiments were carried out to determine if it also acted antagonistically towards the effect of pantothenic acid on the fermentation of deficient yeast. As can be seen in the table below, no inhibition took place when a large quantity of the pantoyl taurine was added either alone or with pantothenic acid. On the contrary, fermentation was accelerated.

Two milligrams of the deficient yeast, suspended in 1 cc. of KH_2PO_4 solution, were added to each flask together with 1 cc. of 0.4M glucose and 0.1 cc. of the substance to be tested. The flasks were allowed to come to equilibrium temperature and the CO_2 evolution was measured.

---

[47] E. E. Snell, J. Biol. Chem., 139: 975 (1941).

[48] E. E. Snell, Ibid., 141: 121 (1941).

Table V

Effect of Pantoyl taurine on the Fermentation of Deficient Yeast

Pantothenic acid added.	Pantoyl taurine added.	mm^3 of CO_2 per hour.
0	0	240 243
1γ	0	240 265
1γ	0.5 mgm.	254
1γ	1 mgm.	278 271
1γ	10 mgm.	296 294
0	1 mgm.	265
0	10 mgm.	297

The effect of pantoyl taurine was compared with that of the pantoic lactone in a similar experiment.

Table VI

Effect of Pantoyl Taurine and Pantoic Lactone on Fermentation by Deficient Yeast

Addition	Mm^3 of CO_2 per hour.
Blank	130 124
2 mgm. of p. t.*	154 127
40 mgm. p. t.	162
10 mgm. lactone	148 138

* p. t. = pantoyl taurine.

The addition of large quantities of pantoic lactone also has an accelerating effect. It seems possible, therefore, that this unexpected effect of the pantoyl taurine was at least partially due to the pantoyl radical. The contrasting behavior of pantoyl taurine on growth of microorganisms and on fermentation is not understood.

IV

EFFECT ON CHICK TISSUES DEFICIENT IN PANTOTHENIC ACID

EFFECT ON CHICK TISSUE DEFICIENT IN PANTOTHENIC ACID

Day old chicks were fed a diet consisting of 74.5% yellow corn meal. 18% washed "vitamin free" casein, and 7.5% salt mixture supplemented by vitamins as follows: liver extract treated with 10% HCl in methanol equivalent to 20% fresh liver in the diet, 0.2 mgm. of thiamin per 100 g. of diet, petroleum ether extract of 1 g. of alfalfa leaf meal per 100 g. of diet, and 2% U. S. P. cod liver oil. This feed had a pantothenic acid content of not more than 0.4 mgm. per 100 g. The experiments were carried out on the tissues when the chicks were three to four months old. The pantothenic acid analyses on the tissues of the deficient chicks and on chicks fed a commercial diet are given below in γ per gm. moist weight. As can be seen, the deficient tissues contained considerably lower content of pantothenic acid than the normal tissues. For all the work reported here the tissues were homogenized according to the method of Potter and Elvehjem.[49].

---

49. V. R. Potter, C. A. Elvehjem, J. Biol. Chem., 114: 495(1936).

Table VII

Pantothenic Acid Content of Chick Tissues

Tissue	Deficient	Normal
Brain	23.2γ	46.2γ
Breast Muscle	3.2γ	12.1γ

Effect on oxygen absorption of breast muscle: The effect of pantothenic acid upon the oxygen absorption of homogenized breast muscle during the utilization of glucose was determined. A control containing no glucose gave considerably less oxygen absorption showing that glucose was being metabolized. It can be seen from Table VIII that pantothenic acid had no effect.

Each flask contained 325 mgm. (moist wt.) of homogenized muscle in 1.5 cc. of phosphate ringer solution without sodium carbonate or calcium ion, 0.3 cc. of 2.5% glucose, 0.1 cc. containing the indicated amount of calcium pantothenate, and 0.15 cc. of 20% KOH in the center cup. An atmosphere of air was used.

Table VIII

Oxygen Consumption of Homogenized Breast Muscle

mm^3 of oxygen per mgm. of tissue (dry wt.)

Calcium pantothenate	5 min	10 min	20 min	30 min	45 min	105 min	120 min
0[a]	0.06	0.11	0.18	0.26	0.36	0.73	0.81
0	0.11	0.17	0.25	0.34	0.45	0.93	1.03
0	0.12	0.18	0.24	0.36	0.44	0.88	0.96
10 γ	0.10	0.15	0.22	0.33	0.44	0.91	0.99
10 γ	0.15	0.20	0.25	0.34	0.43	0.85	0.94

[a] Control, contained no glucose.

Effect on anaerobic glycolysis of brain: The effect of pantoyl taurine acid as well as that of pantothenic acid itself upon the anaerobic glycolysis of pantothenic acid deficient homogenized chick brain was determined. The results shown in Table IX indicate that neither pantothenic acid nor its sulfonic acid homologue has any effect.

The gray matter of the brain from a deficient chick was homogenized in phosphate ringer solution and 2.9 cc. containing 200 mgm. (moist weight) were pipetted into each flask. To this was added 0.1 cc. of water containing the test substance. The flasks were then placed in the bath and aerated for five minutes with a mixture of 95% nitrogen and 5% carbon dioxide. The mixture was passed over

heated copper to remove all traces of oxygen. The sodium D (+) salt of pantoyl taurine was used.

Table IX

Anaerobic Glycolysis of Homogenized Brain

mm^3 of CO_2 per mgm. of tissue (dry wt.)

	15 min.	45 min.	60 min.	Next 15 min.
No pantothenic acid	0.67	2.15	2.56	0.45
10γ calcium pantothenate	0.82	2.13	2.50	0.44
1000γ sodium salt of pantoyl taurine	0.75	2.25	2.25	0.58

A number of other experiments using deficient chick tissue were performed but in no case could any effect of pantothenic acid, either on oxygen consumption or on anaerobic glycolysis be demonstrated. These results indicate that pantothenic acid does not serve as a typical dissociable coenzyme for the glycolytic system. This statement should not, of course, be construed as meaning that pantothenic acid is in no way involved in glycolysis. Rather, in view of previous work it would seem that pantothenic acid must act in a combined form, either tied irreversibly to a protein or in a complex molecule analogous to the thiamin-cocarboxylase relationship.

BIOTIN AND ALCOHOLIC FERMENTATION

BIOTIN AND ALCOHOLIC FERMENTATION

Biotin is present in maceration juice, and a considerable portion (about 0.004γ per cc.) is still present in the acetone precipitated juice. It is possible that this vitamin has a function in carbohydrate metabolism.

It has been shown that avidin, a protein constituent of egg albumen, is capable of binding biotin and rendering it unavailable to yeast and to other organisms[50,51,52]. This binding takes place very rapidly on the addition of egg white to a biotin solution and is not affected by the presence of impurities. If avidin renders biotin unavailable for the growth of yeast it should also tie up any free biotin present in the yeast juice and render it unavailable for action as a coenzyme. This assumption was utilized to determine if biotin serves as a coenzyme for fermentation.

To each Warburg flask was added 1 cc. of acetone treated yeast juice, 0.5 cc. of 0.4M glucose, 0.5 cc. of KH_2PO_4, 0.1 cc. of salt solution, 10γ each of coenzyme I, cocarboxylase, and adenylic acid, 7 mgm. of sodium hexosediphosphate and the indicated amount of egg white dissolved

---

[50] R. E. Eakin, W. A. McKinley, R. J. Williams, Science, 92: 224 (1940).

[51] R. E. Eakin, E. E. Snell, R. J. Williams, J. Biol. Chem., 136: 801 (1940).

[52] P. Gyorgy, C. S. Rose, R. E. Eakin, E. E. Snell, R. J. Williams, Science, 93: 477 (1941)

in water.

Table X

Egg white added	0		0.1 cc. of 1:50		0.5 cc. of 1:50		0.5 cc. of 1:25	
	a	b	a	b	a	b	a	b
Mm^3 of CO_2 per hour	236	222	230	227	202	222	230	199

Eakin[53,54] has shown that egg white contains enough avidin per cc. to render unavailable between 0.8 and 1.2 microgram of biotin. Therefore, all except the lowest dosage used in this test contained sufficient avidin to inactivate all of the biotin.

The failure of avidin to block the fermentation of glucose by maceration juice indicates that biotin is not a dissociable coenzyme for the fermentation system. Three possibilities may be considered.

(1) Biotin is not involved in fermentation.

(2) Biotin is involved in fermentation but is in a combined form from which it cannot be removed by avidin.

(3) The avidin-biotin complex is itself a constituent of the fermentation system.

In order to test this latter hypothesis it is only necessary to test the maceration juice for the presence

---

[53] R. E. Eakin, E. E. Snell, R. J. Williams, J. Biol. Chem., 140: 535 (1941).

[54] R. E. Eakin, Ph. D. Thesis, The University of Texas, (1942).

of avidin. Since heat denatures avidin and frees the biotin, this was done by assaying a heated and unheated sample of the juice.

Table XI

Sample	Biotin Content
Unheated	0.16mγ per cc.
Heated	0.15mγ per cc.

The results show that avidin is not present in yeast juice.

It may be concluded that biotin does not act as a dissociable coenzyme for the fermentation system. These results in no way, however, contravene the possibility that biotin in a combined form plays an important part in fermentation.

SUMMARY

SUMMARY

1. The accelerating effect of pantothenic acid on the fermentation by yeast deficient in pantothenic acid was confirmed.

2. Added pantothenic acid was found to have no effect on the fermentation of glucose when used in conjunction with preparationsof dialyzed yeast maceration juice, or acetone precipitated yeast maceration juice.

3. Addition of pantothenic acid failed to affect the rate of phosphorylation of glucose or the rate of decarboxylation of pyruvic acid by yeast maceration juice.

4. The accelerating effect of pantothenic acid on fermentation by deficient yeast cells was found to be accompanied by a "binding" of pantothenic acid by the yeast cells.

5. Added pantothenic acid had no effect on the rate of glycolysis of homogenized chick tissues deficient in pantothenic acid.

6. Addition of avidin, the biotin binding protein, had no effect on fermentation by yeast maceration juice. The biotin-avidin complex was shown not to be present in the maceration juice.

BIBLIOGRAPHY

BIBLIOGRAPHY

Briggs, A. P., J. Biol. Chem. 59; 255 (1924)

Chambers, W. H., Barker, S. B., Ann. Rev. Biochem. 9; 253 (1940).

Cori, C. F., Cori, G. T., ibid 10; 151 (1941)

Dixon, M., Manometric Methods, London, Cambridge University Press, (1934)

Eakin, R. E., McKinley, W. A., Williams, R. J., Science 92; 224 (1940)

Eakin,R. E., Snell, E. E., Williams, R. J., J. Biol. Chem. 136; 801 (1940)

Eakin, R. E., Snell, E. E., Williams, R. J., ibid 140; 535 (1941)

Eakin, R. E., Ph. D. Thesis, The University of Texas, (1942)

Goda, T., Biochem. Z. 298; 431 (1938)

Gyorgy, P., Rose, C. S., Eakin, R. E., Snell,E. E., Williams, R. J., Science 93; 477 (1941)

Herbert, D., Gordon, H., Subrahmanyan, V., Green, D. E., Biochem. J. 34; 1070 (1940)

Hughes, E. H., J. Nutrition 17; 527 (1939)

Jukes, T. H., J. Amer. Chem. Soc. 61; 976 (1939)

Kerly, M., Bourne, M. C., Biochem. J. 34; 1503 (1940)

Lebedev, A. V., Ann. Inst. Pasteur 26; 8 (1912)

Lutwak-Mann, C., Mann, T., Biochem. Z. 281; 140 (1935)

Macfarlane, M. G., Biochem. J. 33; 565 (1939)

McBurney, E. H., Bollen, W. B., Williams, R. J., Proc. Nat. Acad. Sc. 21; 301 (1935)

McKibbin, J. M., Madden, R. J., Black, S., Elvehjem, C. A., Amer. J. Phys.128; 102 (1939)

McKibbin, J. M., Block, S., Elvehjem, C. A. ibid 130, 3 65 (1940)

Meyerhof, O., Perdigon, E., Enzymologia 8; 353 (1940)

Nord, F. F., Chem. Rev. 26; 423 (1940)

Oleson, J. J., Woolley, D. W., Elvehjem, C. A., Proc. Soc. Exp. Biol. 42; 151 (1939)

Parnas, I. K., Enzymologia 5; 172(1938)

Pennington, D., Snell, E. E., Williams, R. J., J. Biol Chem. 135; 213 (1940)

Potter, V. R., Elvehjem, C. A., J. Biol. Chem. 114; 495 (1936)

Pratt, E. F., Williams, R. J., J. Gen. Physiol. 22; 637 (1939)

Richardson, L. R., Hogan, A. O., Proc. Soc. Exp. Biol. 42; 151 (1939)

Robison, H., Morgan, W. T. J., Biochem. J. 24; 119 (1930)

Snell, E. E., Pennington, D., Williams, R. J., J. Biol. Chem. 133; 559 (1940)

Snell, E. E., ibid 139; 975 (1941)

Snell, E. E., ibid 141; 121 (1941)

Spies, T. D., Stanberry, S., Williams, R. J., Jukes, T. H., Babcock, S. H., J. Amer. Med. Ass. 115; 523 (1940)

Stiller, E. T., Keresztesy, J. C., Finkelstein, J., J. Amer. Chem. Soc. 62; 1779 (1940)

Stiller, E. T., Harris, S. A., Finkelstein, J., Keresztesy, J. C., Folkers, K., ibid 62; 1785 (1940)

Subbarow, Y., Hitchings, G. H., ibid 61; 1615 (1939)

Weinstock, H. H., Jr., Mitchell, H. K., Pratt, E. F., Williams, R. J., ibid 61; 1421 (1939)

Williams, R. J., Bradway, E., J. Amer. Chem. Soc. 53; 783 (1931)

Williams, R. J., Truesdail, J. H., ibid 53, 4171 (1931)

Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., ibid 54, 3462 (1932)

Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., Holaday, D., ibid 55, 2912 (1933)

Williams, R. J., Rohrman, E., Plant Physiol. 10, 559 (1935)

Williams, R. J., Mosher, W. A., Rohrman, E., Biochem. J. 30, 2036 (1936)

Williams, R. J., Rohrman, E., J. Amer. Chem Soc. 58, 695 (1936)

Williams, R. J., Truesdail, J. H., Weinstock, H. H. Jr., Rohrman, E., Lyman, C. M., McBurney, C. H., ibid 60, 2719 (1938)

Williams, R. J., Weinstock, H. H., Jr., Rohrman, E., Truesdail, J. H., Mitchell, H. K., Meyer, C. E., ibid 61, 454 (1939)

Williams, R. J., Major, R. T., Science 91, 246 (1940)

Williams, R. J., Mitchell, H. K., Weinstock, H. H., Jr., Snell, E. E., J. Amer. Chem. Soc. 62, 1784 (1940)

Woolley, D. W., Waisman, H. H., Elvehjem, C. A., ibid 61, 997 (1939)

The vita has been removed from the digitized version of this document.